

REMARKS / ARGUMENTS

Specification Objection

The Examiner noted that trademarks have been used in the specification and requires that they be capitalized and accompanied by the generic terminology. In this amendment, Applicants have added generic terminology to the specification. Also, by virtue of amending the claims to replace the trademarks with the new terminology, Applicants have amended the specification to be consistent with the amended claims. No new matter has been introduced by these amendments.

Applicants, however, disagree with the Examiner that the trademarks must be capitalized. MPEP 608.01(v) states in the Examiner's Note to either capitalize each letter of the word or include a proper trademark symbol, such as TM or [®] following the word. Since Applicants have included a proper trademark symbol where required, Applicants have complied with this provision (see MPEP, Rev. 2, May 2004 at p. 600-88).

Claim Objection

The Examiner has objected to the use of abbreviations for magnesium and calcium in claim 2. Claim 2 has been amended to write out these terms before use of their abbreviations. Applicants therefore request that this objection be withdrawn.

Claim Rejections

35 USC § 112, second paragraph

Claims 1-16 stand rejected because the Examiner alleges that the recitation of trademarks renders the claims indefinite. To overcome this rejection, Applicants have amended the claims by replacing the trademarks with their generic terminology to identify the particular materials. Applicants therefore request that this rejection be withdrawn.

35 USC § 102(e)

Claims 4, 5 and 7-12 stand rejected as allegedly being anticipated by U.S. Patent No. 5, 780, 601 granted to Green et al. ("the Green '601 patent"). Applicants respectfully traverse.

The invention recited in claims 4, 5 and 7-12 is directed to a process for extracting native or recombinantly-expressed, gram-negative outer membrane proteins, such as the lipidated recombinant P4 (rP4) protein of *Haemophilus influenzae*, from bacteria or bacterial host cells by differential detergent tangential flow diafiltration. This process includes the steps of:

- (a) lysing bacteria or bacterial host cells containing a recombinant vector in a fermentation broth;
- (b) diafiltering the lysed fermentation broth from (a) with a buffer which is not retained by the diafiltration membrane, wherein said buffer removes intracellular and extracellular contaminants through the permeate, and using a chelating agent to prevent proteolysis;
- (c) diafiltering the lysate from (b) with a detergent and a buffer which is not retained by the diafiltration membrane, wherein said detergent solubilizes and removes inner membrane proteins, and using a divalent cation to stabilize the outer membrane proteins, thereby preventing their solubilization;
- (d) diafiltering the lysate from (c) with the buffer from (c), and using a divalent cation from (c) in the absence of detergent, in order to reduce the concentration of the detergent from (c);
- (e) diafiltering the lysate from (d) with a buffer which is not retained by the diafiltration membrane, a chelating agent and a detergent to solubilize and remove the outer membrane proteins; and
- (f) collecting the outer membrane proteins removed in (e).

The process of this invention is an improvement over alternate processes, such as centrifugation, in that it provides added selectivity to the process, i.e., not only must the proteins be soluble, they must also have the ability to pass through the diafiltration membrane, which has a defined size cut-off or opening. The sequence of buffer solutions is chosen to solubilize inner membrane proteins first and then to solubilize the outer membrane proteins. During diafiltration, the solubilized proteins of approximate

size less than the molecular weight cut-off of the membrane pass through with the permeate, while larger molecules and unsolubilized proteins are retained.

By contrast, the Green '601 patent discloses nothing about tangential flow diafiltration. Rather, it discloses a method of purifying P4 by differential detergent extraction utilizing methods such as differential sedimentation (col. 4), gradient sedimentation (col. 4), and centrifugation (col. 14), none of which rely on molecular size differences. The only requirement of these processes is that the membrane proteins be either soluble or insoluble; these proteins are not required to pass through a diafiltration membrane. Consequently, since every element of the presently claimed process is not identically shown in the Green '601 patent, the claimed invention cannot be anticipated by it.

Applicants respectfully disagree with the Examiner's contention that it is the Applicants' burden to show a patentable difference between the claimed methods and those of the prior art. Nevertheless, Applicants have in these Remarks demonstrated just such a patentable difference. This rejection is therefore improper and should be withdrawn.

35 USC § 102(b)

Claims 4, 5 and 7-12 stand rejected as allegedly being anticipated by Green et al., *Infection and Immunity*, Sept. 1991, p. 3191-3198 ("Green et al."). Applicants traverse.

The invention recited in claims 4, 5 and 7-12 was described above and will not be repeated here.

Like the Green '601 patent, Green et al. discloses nothing about tangential flow diafiltration. Green et al. does not even disclose how to separate the soluble membrane proteins from the insoluble ones. . . Green et al. cites Zlotnick et al. for this process. "e protein was purified from outer membrane fractions of Hib Eagen prepared as described by Zlotnick et al. (39)." (Green et al., p. 3191, col. 2) Zlotnick et al. (*Journal of Biological Chemistry*, July 15, 1988, p. 9790-9794), in fact, utilized centrifugation to pellet the cell membranes (See Material and Methods, p. 9792).

Since neither Green et al. nor Zlotnick et al. discloses the tangential flow diafiltration process of the presently claimed invention, Applicants submit that this rejection is improper and should be withdrawn.

35 USC § 103(a)

Claims 1-7 and 13-16 stand rejected as being obvious over a combination of references: Anilionis et al. (*U.S. Patent No. 5,098,997*) and Kolbe (*U.S. Patent No. 5,276,141*).

Claims 1-7 and 13-16 are directed to separate processes for extracting native or recombinantly-expressed, gram-negative inner (claims 1-3) and outer (claims 4-7) membrane proteins from bacteria or bacterial host cells containing a recombinant vector by differential detergent tangential flow diafiltration. For inner membrane proteins the process includes the steps of:

- (a) lysing bacteria or bacterial host cells containing a recombinant vector in a fermentation broth;
- (b) diafiltering the lysed fermentation broth from (a) with a buffer which is not retained by the diafiltration membrane, wherein said buffer removes intracellular and extracellular contaminants through the permeate, and using a chelating agent to prevent proteolysis;
- (c) diafiltering the lysate from (b) with a detergent and a buffer which is not retained by the diafiltration membrane, wherein said detergent solubilizes and removes inner membrane proteins, and using a divalent cation to stabilize the outer membrane proteins, thereby preventing their solubilization; and
- (d) collecting the inner membrane proteins removed in (c).

The extraction process for outer membrane proteins in general is described above, and the process for extracting the outer membrane protein P6 is described in claims 13-16.

The process of this invention is an improvement over alternate processes, such as centrifugation, in that it provides added selectivity to the process, i.e., not only must the proteins be soluble, they must also have the ability to pass through the diafiltration membrane, which has a defined size cut-off or opening. The sequence of buffer

solutions is chosen to solubilize inner membrane proteins first and then to solubilize the outer membrane proteins. During diafiltration, the solubilized proteins of approximate size less than the molecular weight cut-off of the membrane pass through with the permeate, while larger molecules and unsolubilized proteins are retained.

The present process has several advantages. First, it combines the clarification and extraction processes into one operation. The membrane proteins are extracted from the cells and separated from cell debris with only one continuous diafiltration process. Second, because the membrane proteins must be able to pass through the diafiltration membrane, they are extracted in a semi-purified state, which simplifies the downstream processing steps. And third, this process avoids the cumbersome process of centrifugation and is therefore more amenable to scale-up.

Anilionis et al. disclose nothing about tangential flow diafiltration. Rather, they disclose a method of isolating and purifying outer membrane proteins P4 and P6 by differential detergent extraction utilizing sonication and centrifugation (col. 26-27), neither of which relies on molecular size differences. As noted above, the only requirement of these processes is that the membrane proteins be either soluble or insoluble; these proteins are not required to pass through a diafiltration membrane. And combining Anilionis et al. with Kolbe does not fill this gap.

Kolbe proposes a process for purifying a highly glycosylated protein from a crude preparation derived from a culture of eukaryotic cells, which process comprises the actions (i) of adding to the preparation a divalent metal ion in a sufficient amount in order to form a mixture which precipitates and (ii) after precipitation, of harvesting the protein from the mixture supernatant (col. 1, lines 22-29).

The Examiner asserts that it would have been obvious at the time the invention was made to add the divalent metal ions as taught by Kolbe to the process of purifying protein as taught by Anilionis et al. to obtain the claimed invention.

Applicants respectfully disagree. It is not the differential detergent extraction steps that make the claimed invention novel and unobvious. Rather, it is the tangential flow diafiltration technology that makes it so. Therefore, focusing on the buffers, detergents, and divalent metal ions common to both the claimed process and those cited in the references misses the point -- that Applicants developed a large scale extraction process that avoids centrifugation and obtains quantities of protein sufficient for economical manufacturing. Therefore, combining the centrifugation process of Anilionis

et al. with the divalent metal ions of Kolbe cannot possibly result in the tangential flow diafiltration process of the claimed invention. Moreover, centrifugation merely separates the soluble from the insoluble; it does not impose upon the membrane proteins the added restriction of having to pass through a diafiltration membrane. Centrifugation is also a sequential, segmented process, whereas the claimed tangential flow diafiltration process provides for continuous extraction of the desired proteins.

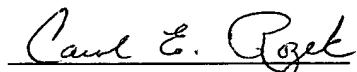
Based on the foregoing, Applicants submit that this rejection based on the combination of Anilionis et al. and Kolbe is improper and should be withdrawn.

Claims 1-7 and 13-16 also stand rejected as allegedly being obvious over the combination of Yang et al. (*Vaccine*, Vol. 15, No. 9, p. 976-987, 1997) and Kolbe (U.S. Patent No. 5,276,141).

Yang et al. likewise disclose nothing about tangential flow diafiltration. As the Examiner points out, they disclose a method of isolating and purifying the outer membrane protein P6 by utilizing centrifugation to form a cell pellet (p. 977-978). Combining the centrifugation process of Yang et al. with the divalent metal ions of Kolbe does not yield the tangential flow diafiltration process of the claimed invention. Thus, this rejection is also improper and should be withdrawn.

In view of the above amendments and remarks, Applicants submit that the present application is in condition for allowance, and a Notice to that effect is requested.

Respectfully submitted,



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